Sequencing of Full-Length cDNA Encoding the α and β Subunits of Human Casein Kinase II from Human Platelets and Megakaryocytic Cells. Expression of the Casein Kinase IIα Intronless Gene in a Megakaryocytic Cell Line†

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ABSTRACT: Casein kinase II (CKII) is a ubiquitous protein kinase composed of two subunits, α and β, that can use both ATP and GTP as phosphoryl donors. Two genes located on two separate chromosomes were identified for CKIIα: one on chromosome 20 band 13 with an approximate size of 20 kb and a second on chromosome 11 band 15.5-p15.4 that is the same size as the cDNA of locus 20 kb (1.2 kb) and does not contain any introns. The two genes differ in four amino acids. Recently, it has been demonstrated that a membrane-associated platelet-derived CKII phosphorylates coagulation factor Va. The mRNA encoding the platelet CKII was isolated from fresh human platelets, and the corresponding cDNAs encoding the α and β subunits of human platelet CKII were produced and sequenced. The cDNA for platelet CKIIα was found to be 99.7% homologous to the CKIIα intronless gene, having the same characteristic amino acid residues at positions 128, 256, 287, and 351. However, the cDNA of platelet CKIIα has a different amino acid at position 236 (Arg → His), which is not found in the intronless gene. The cDNA of the CKIIβ subunit was completely identical with the sequence of the CKIIβ subunit isolated from other tissues. Since platelets arise from megakaryocytes, mRNA was isolated from the megakaryocytic cell line MEG-01 and the cDNA for CKIIα was cloned and sequenced. The cDNA was found to be identical to the intronless gene found in platelets. We have also investigated the expression of the intronless gene in several other cell lines. Expression of the intronless gene was only found in cell line MEG-01. Our data demonstrate expression of the CKIIα intronless gene in megakaryocytes and platelets.

Casein kinase II (CKII) is a pleiotropic, ubiquitous enzyme, which preferentially phosphorylates proteins at serine and threonine residues surrounded by a cluster of acidic amino acid residues (S/TXXD/E) (*1*). Recent results indicate that CKII is also able to catalyze tyrosine phosphorylation (*2*, *3*). CKII is composed of two subunits, α and β. The α subunit occurs in two highly related isoforms, CK2α and CKIIα′. CKII is a tetramer with the α and/or α′ subunit associated stoichiometrically to a regulatory β subunit to form $\alpha_2\beta_2$, or $\alpha'_2\beta_2$, or $\alpha\alpha'\beta_2$ tetramers. The α and α′ subunits possess the catalytic activity and contain the conserved kinase subdomains, whereas the β subunit is believed to be the regulatory subunit (*2*−*4*). It has been demonstrated in *in vitro* reconstitution studies that the catalytic subunit has phosphotransferase activity in the absence of the regulatory (β) subunit and that the presence of the β subunit enhances the activity of the catalytic subunit by 5−10-fold (*5*). CKII has been shown to play a role in cell proliferation, transformation, cell cycle regulation, growth and differentiation, and blood coagulation. A considerable number of proteins have been increasingly identified as substrates for CKII, in particular, proteins involved in blood coagulation. Proteins involved in blood coagulation that are phosphorylated by CKII include coagulation factor V, factor VIII (*6*), factor XI (*7*), fibrinogen (*8*−*10*), vitronectin (*11*), and tissue factor pathway inhibitor (TFPI) (*12*). Thus, while many plasma proteins appear to be substrates for CKII, no CKII kinase activity could be detected in human blood plasma. It is thus possible that plasma CKII may originate from platelets (*13*−*17*). It has been suggested that phosphorylation of factor V/Va and TFPI at Ser^{692} and Ser^{2}, respectively, by a platelet CKII locally at the place of vascular injury may play a vital role in blood coagulation (*18*). CKII phosphorylation of vitronectin at Thr^{50} and Thr^{57} enhances its adhesion to bovine aorta endothelial cells (*11*). The phosphorylation of fibrinogen by CKII was found to have an effect on the structure of the fibrin fibers formed following cleavage of the fibrinogen molecule by α-thrombin (*8*−*10*). CKII α, α′, and β subunits from human platelets have been partially isolated and characterized using specific antibodies (*18*, *19*).

Megakaryocytes, among the rarest of hematopoietic cells, serve the essential function of producing platelets. They are unique when compared with other hematopoietic precursor cells because of their large size and high nuclear ploidy. A number of megakaryoblastic cell lines have been characterized, and each has unique features that distinguish them from bone marrow megakaryocytes (*20*−*25*). However, they can all be induced to undergo further differentiation to varying degrees in the presence of cytokine, growth hormone, and

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phorbol esters, such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (*23*−*25*).

The cDNA for CKIIα is 1.2 kb long. Mapping of human chromosomes through in situ hybridization demonstrated the existence of two genes for the CKIIα subunit; one is located on chromosome 20 band 13 that has an approximate size of 20 kb. The second gene is located on chromosome 11 band 15.5-p15.4 and is the same size as the cDNA of the locus containing the 20 kb gene (1.2 kb). The promoter for the former gene shows the characteristic of a housekeeping gene: a high GC content, the presence of several GC boxes, and the lack of a TATA box. The latter intronless gene of 1.2 kb has the typical features of a processed gene such as the absence of introns, a short poly(A) tail, and direct flanking repeats (*26*−*29*). However, it has a strong promoter with two TATA boxes and a CAAT box upstream from the potential initiation codon. The DNA sequence is 99% homologous to the cDNA derived from the gene contained within locus 20, including the complete coding region of 1170 bp and 150 bp 5′ and 171 bp 3′ untranslated regions (*27*−*29*). To date, no evidence for the expression of the intronless gene located on locus 11 has yet been demonstrated (*27*−*29*). When compared to the cDNA sequence of the 20 kb gene, the processed pseudogene has four characteristic nucleotide substitutions within the coding region at positions 1621, 2004, 2097, and 2288, resulting in Leu^{128} → Phe, Asp^{256} → Gly, Ser^{287} → Arg, and Met^{351} → Val amino acid substitutions. Two additional nucleotide substitutions do not result in a substitution of the corresponding amino acid residues (*27*). These four amino acid substitutions distinguish the coding region of the intronless gene located on chromosome 11 from the gene located on chromosome 20.

In the study presented here, we have cloned and sequenced the cDNA for CKIIα and CKII*β* subunits using RNA isolated from fresh human platelets. We have also investigated the expression of the CKIIα gene in a megakaryocytic cell line (MEG-01), an erythroleukemic cell line (HEL), and a nonhematopoietic carcinoma cell line (HeLa).

EXPERIMENTAL PROCEDURES

Materials. The RNA RT-PCR miniprep kit was purchased from Stratagene (La Jolla, CA). SuperScript RNA reverse transcriptase, Platinum Taq DNA polymerase, ribonuclease H, oligo(dT), DNA gel ladders, DNA primers, and the pGEMT vector were from Gibco BRL Life Technologies/Invitrogen (Carlsbad, CA). The Qiaquick gel extraction kit and the Qiagen plasmid mini kit were obtained from Qiagen (Valencia, CA). The GeneElute Mammalian Genomic DNA kit was purchased from Sigma (St. Louis, MO). General chemicals and reagents were analytical grade.

Platelet Preparation. Human platelets were prepared from freshly drawn blood from consenting donors and isolated as previously described (*30*). Fresh platelet preparations were provided by T. Ugarova from the Department of Cardiology at the Cleveland Clinic Foundation.

Cell Culture. MEG-01 and HEL cells (generously provided by P. Tracy, Biochemistry Department, College of Medicine, University of Vermont, Burlington, VT) were grown in RPMI medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, penicillin/streptomycin, 2 mM L-glutamine, and 4.5 g/L glucose. HeLa cells (generously provided by A. Zhou, Department of Chemistry, Cleveland State University) were grown in DMEM medium supplemented with 10% (v/v) fetal bovine serum and penicillin/streptomycin. Cells were maintained in an incubator (37 °C) with an atmosphere containing 5% CO_2.

Isolation of Total RNA from Human Platelets and MEG-01, HEL, and HeLa Cell Lines. High-quality and DNA free total RNA was isolated from the cells using the RNA RT-PCR miniprep kit according to the manufacturer's instructions. Briefly, 10^5 cells were lysed in a buffer containing guanidine thiocynate and prefiltered in a spin cup. The filtrate was loaded on a spin cup with a silica-based fiber matrix where RNA binds to the fiber matrix. Bound RNA was washed with low-salt buffer and treated with Dnase I for 20 min at 37 °C. Following Dnase I treatment, the RNA was washed with high-salt and low-salt buffer and finally eluted with 50 μL of elution buffer. RNA was quantified spectrophotometrically at 260 nm. To assess the quality and integrity of the RNA, the molecule was visualized on a formaldehyde−agarose gel. The major contaminants in the platelet preparations are usually leukocytes. To ascertain that no contaminating leukocytes were present in our platelet preparations, we performed RT-PCR using primers for the I domain of the glycoprotein receptor Mac-1 (CD11b/CD18). This receptor is specific for leukocytes (*31*−*33*). The primers that were used (generously provided by E. Plow, Department of Molecular Cardiology, Cleveland Clinic Foundation) were as follows: forward, 5′-CTGTTTGGATCCAACCTACG-GCAGCAGC-3′; and reverse, 5′-GGGGCCATTAGAGGT-GATCTCGAGGCTGAAGC-3′.

Genomic DNA Isolation. Genomic DNA was isolated from ∼10^5 MEG-01 cells using the GeneElute Mammalian Genomic DNA kit (Sigma) according to the manufacturer's instructions.

PCR Primers. CKII subunit specific primers, which are complementary to the 5′ and 3′ end of the cDNA encoding the CKIIα and CKII*β* subunits encompassing the stop codon, were used. For the CKIIα subunit, the primers were as follows: P_1, 5′-GCCATATTGTCTGTGTGAGC-3′ (residues 1067−1086, sense); and P_2, 5′-CGTTACTGCTGAGCGC-CAGCGGC-3′ (residues 2415−2393, antisense) (Figure 1). For the CKII*β* subunit, the primers were as follows: P_3, 5′-GCGATGAGCAGCTCAGAGGAGGT-3′ (sense); and P_4, 5′-TTAGCGAATCGTCTTGACTGG-3′ (antisense). An intronless gene specific primer P_0 for the CKIIα subunit was also used [5′-GACTCTCAGATTAGAGATGGGAAGG-3′ (residues 1000−1025, sense)] (Figure 1). Primers P_5 [5′-TCCATATCTTACCGTGCTTCT-3′ (residues 4−25, sense)] and P_6 [5′-TGAGCTTAAATGTCATAGGG-3′ (residues 2611−2631, antisense)] which are complementary to the intronless gene upstream and the 3′ end region were used to amplify the gene from the upstream promoter region up to the end of the coding region when the MEG-01 genomic DNA was used as a template. Nucleotide position numbers are based on the published sequence of the CKIIα gene (*34*).

*RT-PCR and cDNA Synthesis for CKIIα and CKII*β *Subunits*. The cDNA was synthesized from 1 μg of isolated RNA in a total reaction volume of 20 μL containing 500 ng of oligo(dT) primer, 1 μL of a 10 mM dNTP mixture, and 200 units of reverse transcriptase at 37 °C for 50 min. Reverse transcriptase was inhibited by heating the reaction mixture at 70 °C for 20 min. Finally, RNA was digested with 2 units of ribonuclease H, following incubation at 37

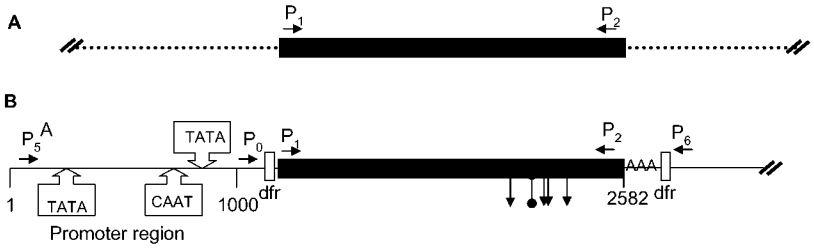

FIGURE 1: Location of PCR primers (P) and amino acid substitutions in the CKIIα gene: (A) gene from locus 20 and (B) the processed gene (intronless gene). Homologous regions are represented by the solid bar, and the regions specific to the processed gene are represented by the thin line in panel B. The downward pointing arrows represent the amino acids substitutions in the processed gene of platelets or megakaryocytic cDNA, compared to the normal gene. Blunt head bars represent the amino acids substitutions found only in the cDNA of platelets or megakaryocytic CKIIα. The abbreviation dfr stands for direct flanking repeats.

°C for 20 min. The cDNA (2 μL) was used for PCR amplification with high-fidelity Taq polymerase. PCRs were programmed as follows: denature at 94 °C for 15 s, anneal at 55 °C for 30 s, and extend at 68 °C for 1 min/kb in a GeneAmp PCR System 9700 DNA thermal cycler (Perkin-Elmer Biosystems, Foster City, CA); specific primers (0.2 mM) were used for the amplification of the corresponding cDNA. PCR products were analyzed following electrophoresis on a 1% agarose gel. Parallel control experiments without the addition of reverse transcriptase were performed to verify possible DNA contamination in the RNA samples.

Sequencing and Cloning of cDNA for CKIIα and CKIIβ Subunits. cDNA bands of the expected size were extracted from agarose gels using the Qiagen gel extraction kit. Platelet CKIIα and CKII*β* cDNA were subcloned into the pGEMT plasmid vector. cDNAs were sequenced in a Beckmen automatic DNA sequencer using universal primers T7 and SP6 and other sequencing primers in the sequencing facility of S. Yadav at the Cleveland Clinic Foundation. The cDNAs for CKIIα synthesized from MEG-01, HEL, and HeLa cells were subcloned into the pGEMT plasmid vector or directly sequenced from the PCR product. DNA primers complementary to every 250−300 bp segment were used for DNA sequencing to verify the accuracy of the sequencing. The cDNA sequences were translated in the corresponding amino acid sequences and compared with other sequences using the BLAST software from the National Center for Biotechnology Information.

RESULTS

Isolation and Sequence of cDNA for CKIIα and CKIIβ from Fresh Human Platelets. To identify the transcripts for CKIIα and CKII*β* in human platelets and megakaryocytes and to investigate their expression, we used the reverse transcriptase polymerase chain reaction (RT-PCR), which is more sensitive than Northern blot analysis. To detect the transcript of the CKIIα subunit intronless gene alone, an intronless gene specific sense primer (P_0) was used (Figure 1). Another primer, P_1, detected both the intronless gene and the gene located on chromosome 20. RT-PCR of the platelet CKIIα subunit using primers P_1 and P_2 (Figure 1), corresponding to the α subunit of CKII, produced a DNA band of 1348 bp (Figure 2, lane 3), while a band of 670 bp was observed using primers P_3 and P_4, corresponding to the amplification of the *β* subunit of CKII (Figure 2, lane 5). RT-PCR using the 5′-end intronless gene specific primer P_0 and P_2 did not lead to detection of the DNA band of the expected size when using the RNA isolated from platelets (Figure 2, lane 2). When the RT-PCRs were performed

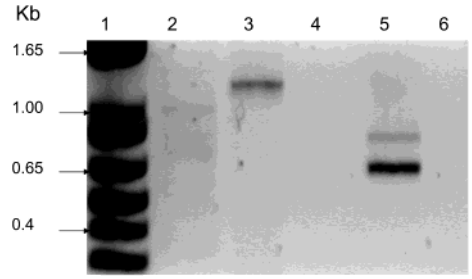

FIGURE 2: RT-PCR profile of human platelet CKII subunits. Total platelet RNA was isolated, and RT-PCR was performed as described in Experimental Procedures. At the end of the reaction, 5 μL of each sample was loaded onto a 1% agarose gel: lane 1, DNA markers; lane 2, P_0 and P_2 α subunit processed (gene specific); lane 3, P_1 and P_2 common primers for the α subunit sequence; lane 4, P_1 and P_2 RT-PCR without the addition of reverse transcriptase (control); lane 5, P_3 and P_4 *β* subunit; and lane 6, P_3 and P_4 RT-PCR without the addition of reverse transcriptase (control).

without the addition of reverse transcriptase, no DNA bands were observed (Figure 2, lanes 4 and 6). Finally, RT-PCR using primers specific to the I domain of Mac-1 (CD11b/CD18), which is a specific receptor only found on blood leukocytes (*31*−*33*), did not reveal any additional band, suggesting that no contaminating leukocytes were present in our platelet preparation (data not shown). Comparison of the nucleotide sequence and the deduced amino acid sequence of platelet CKII α and *β* subunits with the existing consensus sequence in the data bank was carried out using the GenBank nucleic acid data bank and the Protein Identification Resource protein data bank.

The sequencing data of the DNA band of 1348 bp demonstrated that 89% of the clones obtained using platelet RNA and primers P_1 and P_2 had a sequence that was 99.7% similar to the sequence of the human CKIIα subunit (GI no. 599777). This sequence corresponds to the CKIIα intronless gene that was previously isolated from whole blood DNA (*27*). There are two nucleotide differences between our sequence from the platelet-derived cDNA and the DNA sequence of the previously described intronless or processed gene (Figure 3). The platelet cDNA for the CKIIα intronless gene contains a T → G substitution and an A → G substitution at positions 1768 and 1944, respectively. The substitution at nucleotide 1768 does not result in a change in an amino acid. However, the nucleotide substitution at position 1944 results in an amino acid substitution at position 236 (Arg → His). The cDNA sequence of the platelet CKII*β* subunit was identical to the previously published sequence of the human CKII*β* subunit (*35*). No amino acid substitu-

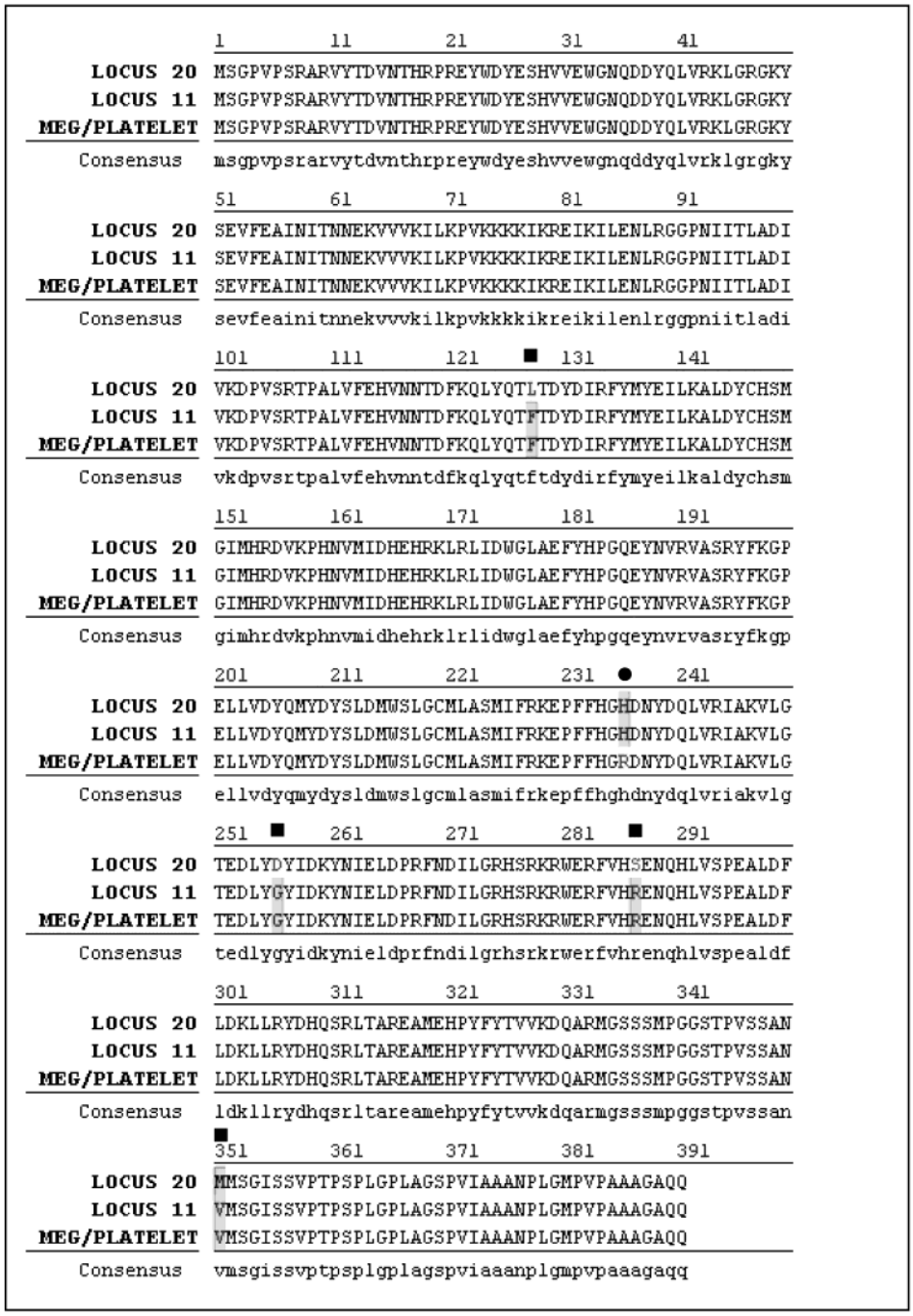

FIGURE 3: Comparison of the amino acid sequences for the casein kinase II α subunit. The amino acid sequences derived from the cDNA of the gene located on chromosome 20 (locus 20), the intronless gene (locus 11), and the platelet- and megakaryocytic cell line-derived (MEG-01) cDNA were compared. Black squares represent the amino acid residue substitutions in both the CKIIα intronless gene and the gene from the MEG-01 cell line. The black circle represents the amino acid residue which is specific to the sequence found in MEG-01 and platelets alone.

tions in the nucleotide sequence of the CKII*β* subunit were found. These data demonstrate that platelets contain the mRNA for CKIIα and CKII*β*. Our data also demonstrate that platelets contain the transcript encoding a functional CKIIα subunit which derives from the intronless gene. To avoid false results in the RT-PCR due to genomic DNA contamination in our RNA preparation, several commercially available kits for RNA isolation were tested. Similar results were obtained each time. To control for DNA contamination in our RNA preparation, we used DNAse, and for each RT-PCR, a control reaction without reverse transcriptase was also performed. The nucleotide and derived amino acid sequences of platelet/megakaryocyte α and *β* subunits were deposited in GenBank (accession numbers AY112721 for CKIIα and AY113186 for CKII*β*).

Isolation and Sequence of cDNA Encoding CKIIα from a Megakaryocytic Cell Line. To verify whether the precursor cells of platelets express the CKIIα intronless gene, RNA was isolated from MEG-01, HEL, and HeLa cells and used for RT-PCR. RNA isolated from HeLa cells, which do not express the intronless gene (*28*), was used as a control. RT-PCR using primers P_0 and P_2 (P_0 is the CKIIα intronless gene specific primer) or primers P_1 and P_2 (Figure 1) and RNA isolated from MEG-01 cells resulted in DNA bands

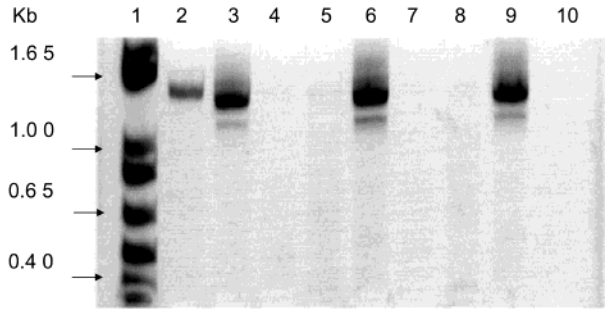

FIGURE 4: RT-PCR profile of two megakaryocytic cell lines and a nonmegakaryocytic cell line. Total platelet RNA was isolated from human platelets and MEG-01, HEL, and HeLa cell lines. RT-PCR was performed as described in Experimental Procedures. At the end of the reaction, 5 μL of each sample was loaded onto a 1% agarose gel: lane 1, DNA markers; lane 2, MEG-01 P_0 and P_2 primers; lane 3, MEG-01 P_1 and P_2 primers; lane 4, MEG-01 P_1 and P_2 RT-PCR without the addition of reverse transcriptase (control); lane 5, HEL P_0 and P_2; lane 6, HEL P_1 and P_2; lane 7, HEL P_1 and P_2 RT-PCR without the addition of reverse transcriptase (control); lane 8, HeLa P_0 and P_2; lane 9, HeLa P_1 and P_2; and lane 10, HeLa P_1 and P_2 RT-PCR without the addition of reverse transcriptase (control).

of 1415 or 1348 bp, respectively (Figure 4, lanes 2 and 3). However, in HEL and HeLa cell lines, only the DNA band of 1348 bp was detected. The DNA band of 1415 bp (P_0 and P_2) was not seen in either of these two cell lines (Figure 4). DNA sequencing analysis of the 1415 bp band (P_0 and P_2) from the MEG-01 cell line showed that the sequence encoding CKIIα was identical to the cDNA of platelets (which is 99.7% similar with the CKIIα intronless gene sequence). Sequencing of the DNA bands of 1348 bp that were detected using RNA isolated from HEL and HeLa cells and P_1 and P_2 primers (Figure 4, lanes 6 and 9) revealed complete identity to the sequence of CKIIα derived from the gene located on chromosome 20. No sequence similarity to the intronless gene was detected in the HEL and HeLa cell lines. Any primer complementary to and beyond the 3′ end second poly(A) tail of the intronless gene used for cDNA synthesis did not result in any DNA synthesis (data not shown). Control RT-PCRs without the addition of reverse transcriptase did not result in any DNA synthesis (Figure 4, lanes 4, 7, and 10). To verify the promoter region of the platelet/megakaryocytic CKIIα intronless gene, genomic DNA was isolated from MEG-01 cells and PCR was performed using intronless gene specific 5′ end and 3′ end primers (primers P_5 and P_6, Figure 1). Sequence analysis of the corresponding DNA band showed that the coding region was 100% identical to the cDNA of CKIIα derived from the intronless gene synthesized from platelets or MEG-01 cells. The sequence of the promoter region was found to be identical to the sequence of the promoter region of the CKIIα intronless gene published previously (*26*−*29*). The data demonstrate that platelets and megakaryocytes contain an intronless gene that encodes CKIIα.

DISCUSSION

Our findings indicated that in platelets the CKIIα subunit is encoded by an intronless gene, which is expressed in the megakaryocytic MEG-01 cell line. This gene is different from the gene encoded by chromosome 20 and is characterized by four different amino acids that are unique to the intronless gene.

The gene for the CKIIα subunit that is located on chromosome 11 band 15.5-p15.4 is a processed gene that is formed by retrotransposition which is characterized by the absence of introns, the presence of flanking direct repeats, and a 3′ polyadenylation tail. There are a number of processed genes in the human genome as well as in the genome of other living organisms. To date, 189 processed genes from chromosomes 21 and 22 have been identified and analyzed. The predicted number of processed genes in the entire human genome is estimated to be ∼10000 (*36*). Some of the processed genes are expressing actively, and such functionally processed genes are termed intronless genes (for a review, see ref *37*). Some important intronless genes include genes encoding cyclin D2 which is expressed in human ovary cells in a tissue and age specific manner (*38*), steroid 21-hydroxylase (CYP21P) which is specifically transcribed in the adrenal cortex (*39*), phosphoglycerate mutase brain isoform (*PGAM* 1, *PGAM* B) (*40*), human serotonin 5′-hydrotryptamin receptor (*41*), and the h-Scot-t intronless gene specifically expressed in the testis (*42*). Occasionally, a transcript of an intronless gene can be more prevalent than the transcripts of a gene possessing introns (*37*); e.g., the intronless gene of the tumor suppressor gene *PTEN* is the predominant transcript in liver and glioblastoma cell lines but represents a minor species in spleen and kidney (*43*). As mentioned earlier, the CKIIα intronless gene identified in this study has a strong promoter with two TATA boxes and a CAAT box upstream from the potential initiation codon, a short poly(A) tail, and direct flanking repeats. In this gene, all introns are precisely removed. There is no stop codon or deletion in the open reading frame, which would result in the production of a truncated protein, or no protein. In fact, in the mouse genome, there are three intronless genes for CKIIα. Two of them encode an incomplete protein, while the third one encodes the full-length protein (*44*, *45*).

Our findings show that 89% of the clones containing the cDNA for CKIIα which was synthesized from mRNA isolated from fresh human platelets encode the CKIIα intronless gene. These findings, which are based on the four amino acid differences between the intronless gene and the gene located on locus 20, indicate that human platelets contain CKIIα transcripts for both genes. However, the transcript of the intronless gene is the predominant one in human platelets. The platelet cDNA for the CKIIα intronless gene contains a T → G substitution and an A → G substitution at positions 1768 and 1944, respectively, when compared to the DNA sequence of the intronless gene isolated from other tissues (*27*−*29*). The nucleotide substitution at position 1944 results in an amino acid mutation at position 236 (Arg → His). Using platelets collected from nine different normal donors, the same substitutions at these two positions were observed. At present, we do not have an explanation for the discrepancy between our data and the previously published sequence of the intronless CKIIα gene. Moreover, the CKIIα cDNA obtained by using the mRNA from the MEG-01 cell line has the same nucleotide substitutions at these two positions in addition to the substitutions encoding the four different amino acids specific to the intronless gene. However, none of these amino acid substitutions in the platelet-derived cDNA or the DNA sequences of the CKIIα intronless gene are in the conserved region of the CKIIα subunit (*46*) and/or in the protein kinase domain of the enzyme (*47*). Thus, these five amino acid substitutions

within CKIIα will most likely not affect the function of the protein. As a consequence in platelets and megakaryocytes, the intronless transcript which contributes 89% of the total CKIIα transcripts may have a physiologically relevant function and may be exclusively responsible for the synthesis of the CKIIα subunit. The inability of the specific primer (P_0) directed to the 5′ end of the processed gene to detect the cDNA of the intronless gene in platelets might be due to partial degradation or modification of the mRNA in platelets since platelets are anucleated cellular fragments that arise from megakaryocytes.

The RNA present in platelets derives from megakaryocytes during platelet transformation. Our investigations in the megakaryocytic MEG-01 cell line further validate the observation of a functional CKIIα intronless gene since the transcript detected in MEG-01 cells encodes a CKIIα subunit which has a sequence similar to the one found in platelets. We could not detect the processed gene transcript in HEL cells (an erythroblastic cell line) or in HeLa cells. Our data with the HeLa cell lines support the previous observation of Wirkner and Pyerin (*28*), where no activity of the promoter for the processed gene was detected in these cells in the promoter assay experiment. Previous work has suggested the probable expression of this gene in certain tissues or cells (*27*−*29*). DNA sequence analysis of the upstream region of the CKIIα intronless gene isolated from the genomic DNA of MEG-01 indicated that the intronless gene has a strong promoter region, including two TATA boxes and a CAAT box upstream from the potential initiation codon. On the contrary, the CKIIα gene encoded by chromosome 20 (locus 20) has housekeeping-like promoter elements.

In conclusion, the CKIIα intronless gene might have an advantage over the normal CKIIα gene in the megakaryocytes and platelets in which sophisticated lineage specific genes expression is necessary during cell proliferation and/or differentiation.

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